Docket NO.: 62,738A PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
Tatsuji SEKI et al.	:	
Serial No. 09/857,651	:	Group Art Unit: 1638
Filed: August 27, 2001	:	Examiner: D. Kruse

For: METHOD FOR MANUFACTURING GLYCOPROTEINS HAVING HUMAN-TYPE GLYCOSYLATION

DECLARATION UNER 37 CFR §1.132
IN RE KATZ DECLARATION

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

1. We, Tatsuji SEKI and Kazuhito FUJIYAMA are co-authors of the publication: "Transformation of Tobacco Using Human β-1,4-galactosyltransferase Gene and Regeneration of Transgenic Plants," Annual Reports of IC Biotech, Vol. 18, 1995, publicly available August 31, 1998, p. 241-247.

2. We are also co-inventors of the U.S. Patent Application Serial No. 09/857,651 which was filed August 27, 2001 with claims directed to METHOD FOR MANUFACTURING GLYCOPROTEINS HAVING HUMAN-TYPE GLYCOSYLATION.

3. We further declare that the remaining co-authors of the publication "Transformation of Tobacco Using Human β-1,4-galactosyltransferase Gene and Regeneration of Transgenic Plants," Annual Reports of IC Biotech, Vol. 18, 1995, p. 241-247 publication, while collaborating scientifically and being co-authors of the above referenced publication, were not co-inventors of the subject matter disclosed and claimed in the U.S. Patent Application Serial No. 09/857,651.

4. Yi Zhang worked as a student in our laboratory under our direction and supervision.

5. Toshiomi Yoshida was the director of the International Center for Biotechnology and his work was not directly related to this invention.

6. We further declare that the remaining co-authors of the above referenced "Transformation of Tobacco Using Human β-1,4-galactosyltransferase Gene and Regeneration of Transgenic Plants," Annual Reports of IC Biotech, Vol. 18, 1995, p. 241-247 publication did not contribute to the conception of the invention.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Feb. 28, 2005

Tatsuji SEKI

Date: Feb/28/'05

Kazuhito FUJIYAMA

Docket No.: 62,736[illegible] PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
Tatsuji SEKI et al.	:	
Serial No. 09/857,651	:	Group Art Unit: 1638
Filed: August 27, 2001	:	Examiner: D. Kruse

For: METHOD FOR MANUFACTURING GLYCOPROTEINS HAVING HUMAN-TYPE GLYCOSYLATION

DECLARATION UNER 37 CFR §1.131

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

We, Tatsuji Seki and Kazuhito Fujiyama, declare and say as follows:

1. We are named co-inventors on the above-identified application.

2. Attached hereto are Exhibits 1-6 which represent copies of consecutive pages taken from Professor Kazuhito Fujiyama's laboratory notebook.

3. All work represented by the laboratory notebook entries contained in Exhibits 1-6 was performed in Japan after January 1, 1996 but before April 20, 1998. Dates which were entered in the left-hand margin of the original laboratory notebook have been redacted from the copies.

4. Exhibit 1 describes a partial protein purification. The starting material for the purification was two cultivated BY-2 tobacco callus cell samples, a negative control designated wild-type (WT), consisting of a single transformant containing a C1a Peroxidase gene (which is a horseradish peroxidase isozyme gene), and a double transformant designated GT6 (later, simply called GT) containing the C1a horseradish peroxidase gene plus a human galactosyltransferase gene. Sample cells were ruptured with a mortar and pestle in the presence of liquid nitrogen. The broken cells were suspended in a 50mM Tris-HCl buffer (pH 6.8) and centrifuged. Ammonium sulfate was added to each resulting supernatant followed by incubation on ice and centrifugation. Supernatants were collected and ammonium sulfate was again added to each supernatant followed by centrifugation. The precipitate was collected, suspended in a Tris-HCl buffer (pH 6.8) and dialyzed three times in 6/36 inch dialysis tubing against the Tris-HCl buffer to remove the ammonium sulfate and to redissolve the precipitate.

5. Exhibit 2 describes, at the top of the page, a buffer for a concanavalin A (ConA) column containing 10 mM Tris-HCl (pH 6.8), 0.15 M NaCl, 1mM $MgCl_2$, 1Mm $CaCl_2$, and 0.1 mM $MnCl_2$. Thereafter, the results of an assay for peroxidase (POD) activity are described for 1:100 and 1:1000 dilutions of each of the dialyzed supernatants described in Exhibit 1 from the single transformant (WT) and the double transformant (GT). The WT sample gave an absorbance of 0.617 and the WT sample gave an absorbance of 0.217 at 480 nm, thereby confirming that both protein preparations contained peroxidase activity. Exhibit 2 additionally describes a further purification of the two partially purified protein samples on ConA affinity columns. Each of the dialyzed supernatants was loaded separately onto a 2 ml ConA column to

bind the HRP glycan's mannose subunits (0.5 ml GT dialyzed supernatant and 0.18 ml WT dialyzed supernatant, made up to 0.5 ml with 50 mM Tris-HCl, pH 6.8). The ConA columns were washed with the buffer and fifteen 1.5 ml fractions (tube nos. 1-15) were collected for each sample. The glycoprotein samples were then eluted with the ConA buffer containing 0.2 M methyl-α -D-glucoside in a further fifteen 1.5 ml fractions (tube nos. 16-30).

6. Exhibit 3 shows the results of an enzyme assay that was performed on each fraction of each sample that was eluted from the ConA column in accordance with the table and the chromatograph. The purpose of this assay was to determine which of the samples contain HRP. The enzyme assay used a mixture containing Solutions A (1 ml), B (1 ml) and C (2 ml) which were prewarmed to 25°C for 5 minutes. Thereafter, an enzyme solution (0.5 ml) was added to the mixture. The resulting mixture was incubated at 25°C for 3 minutes and then the reaction was stopped by the addition of 0.1 N HCl (0.5 ml). Absorbance for each fraction of each sample was detected at 480 nm. As can be seen from Exhibit 3, Tube no. 17 and no. 18 for both the single transformant WT and double transformant GT samples showed HRP activity. These fractions were pooled for each sample, and dialyzed twice against 10 mM Tris-HCl (pH 6.8). After dialysis, the HRP enzyme assay was performed once again. The GT and WT samples gave an absorbance of 0.363 and 0.221, respectively, at 480 nm.

7. Exhibit 4 shows further purification of each of the dialyzed pooled fractions (WT 0.18 ml; GT 0.50 ml) on an RCA-120 column. Specifically, each sample was loaded onto a separate column to bind the glycoproteins containing galactose terminal residues. The columns were then washed with 20 mM Na-phosphate buffer (pH 7.0) containing 0.15 M NaCl, and twelve 1.5 ml fractions were

collected (tube nos. 1-12). The glycoproteins were then eluted with a buffer (20 mM Na-phosphate, pH 7.0), containing 0.15 M NaCl and 0.2 M lactose, in a further twelve 1.5 ml fractions (tube nos. 13-24). The set of 12 tubes containing the lactose buffer eluent was run in an HRP enzyme assay with the same reagent mixture A, B, and C.

8. Exhibit 5 contains the results of the HRP enzyme assay. Tube no. 13 and no. 14 of the WT sample showed no activity, while tube no. 13 and no. 14 of the GT sample each showed an HRP activity resulting in an absorbance of 0.024 and 0.013, respectively, at 480 nm. These enzyme fractions from tube no. 13 and no. 14 were subsequently pooled, dialyzed against water and freeze-dried.

9. Exhibit 6 shows a certificate of analysis for a *Ricinus communis* A-linked Agarose (RCA-120) resin, the lectin-resin that was used for the subsequently described affinity chromatography.

10. The purification of glycoproteins with peroxidase activity by binding on an RCA-120 lectin column in the double transformant cells indicated that these cells transferred a β 1,4-linked galactose residue to the non-reducing terminus of the HRP glycan. The failure of HRP from the single transformant to bind to the RCA-120 lectin column showed that plant cells without the human galactosyltransferase gene were unable to produce a glycan with affinity to the lectin, that is, with galactosylated glycans.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Feb/28/'05

Kazuhito FUJIYAMA

Date: Feb. 28. 2005

Tatsuji SEKI